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RAPID SCREENING METHOD FOR RADIATION BIODOSIMETRY

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ABSTRACT

The dicentric chromosome assay (DCA) is the 'gold'-standard assay for accurately estimating unknown radiological doses to individuals following radiological or nuclear accidents. However in a mass casualty scenario, the conventional DCA is not well suited for providing timely dose estimates due to the time- and expertise-intensive nature of this assay. In Canada, two approaches are being developed in an attempt to increase triage-quality biological dosimetry in a mass casualty scenario. These are: 1) increasing the number of trained personnel capable of conducting the DCA and, 2) evaluating alternative biodosimetry approaches or DCA variations, such as decreasing the number of metaphase cells scored. In a recent exercise, a new scoring technique (termed DCA QuickScan) was evaluated as an alternative rapid scoring approach. Conventional DCA and DCA QuickScan analysis was based upon scoring a minimum of 50 metaphase cells or 30 dicentrics by 9-15 scorers across 4 laboratories. Dose estimates for the conventional DCA were found to be within 0.5 Gy of the actual dose for 83% of the unknown samples, while DCA QuickScan dose estimates were within 0.5 Gy for 80% of the samples. Of the dose estimates falling 0.5 Gy or more outside the actual dose, the majority were dose over-estimates. It was concluded that the DCA QuickScan approach can provide critical dose information at a much faster rate than the conventional DCA without sacrificing accuracy. Future studies will further evaluate the accuracy of the DCA QuickScan method.

Keywords: dicentrics; chromosome aberrations; biodosimetry; triage; cytogenetics; emergencies, radiological; accidents, nuclear

INTRODUCTION

Over the past 60 years, scientists have been working towards establishing biological methods that would be able to accurately predict unknown radiation doses received by irradiated individuals. In 2004, the International Organization for Standardization (ISO) accepted the DCA as an International Standard and published guidelines (International Organization for Standardization (ISO) 2004) for service laboratories performing radiation biological dosimetry by cytogenetics. Experience with the DCA in the evaluation of hundreds of cases of suspected or verified radiation over-exposures throughout the world has demonstrated the usefulness of this technique for the purpose of providing personal dose estimates in the absence of physical dosimetry (Lloyd et al. 2001; Padovani et al. 1993; Thierens et al. 2005; Voisin et al. 2001; Voisin et al. 2004; Wojcik et al. 2004).

One of the biggest limitations of the conventional DCA assay for use in emergency biological dosimetry is the extensive time and expertise required to perform the scoring. Traditionally, 500 to 1000 metaphase spreads are analyzed for each sample to provide accurate biological dosimetry in cases where only a small number of dose estimates are required. In these cases, it is feasible to score up to 1000 metaphase spreads per sample, resulting in a sensitivity of 0.15 to 0.20 Gy. However, this process is extremely labour intensive and time consuming, requiring three to four days to produce the slides and at least five days for one microscopist to score one sample. In the case of a large-scale radiological event, where potentially thousands of people could have been exposed to radiation, biological dosimetry using the conventional DCA is not feasible. Lloyd et al. (2000) suggested that the conventional DCA may still play a role in the early stages of medical management (triage) by decreasing the number of metaphase spreads analysed, yet maintaining sensitivity to detect clinically relevant doses. In a mass casualty event, it is generally agreed that only those individuals receiving a whole body equivalent dose of more than 1.5 Gy would require any medical intervention (Alexander

et al. 2007). In order to achieve that level of sensitivity, scoring only 50 metaphase spreads (or 30 dicentrics) would be required and would provide biological dose estimates within 0.5 Gy (Lloyd et al. 2000). Recently the ISO also published a standard for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear events (ISO 2008).

In Canada, through funding provided by the Chemical, Biological, Radiological- Nuclear and Explosives (CBRNE) Research and Technology Initiative (CRTI), a strategy is being developed to increase the throughput for the DCA for emergency purposes under the National Biological Dosimetry Response Plan (NBDRP). The network, formally called the Cytogenetic Emergency Network (CEN), is currently comprised of four core laboratories (Health Canada-Ottawa (HC), Atomic Energy of Canada Limited-Chalk River Laboratories (AECL), Defence Research and Development Canada-Ottawa (DRDC) and McMaster University-Hamilton (MU) that are capable of providing radiation biological dose estimates using the conventional DCA (Miller et al. 2006).

The aim of the present study was to determine the current capacity, efficiency and accuracy of the CEN by evaluating the ability of all scorers within each laboratory to accurately assess dose in an emergency scenario. As a second objective, new DCA scoring strategies were investigated that could be used as initial screening tools, namely scoring fewer cells or scoring according to a modified criteria (termed DCA QuickScan).

MATERIALS AND METHODS

Scenario

The scenario for this exercise, initiated on 7 December 2007, involved a radioactive package being received at a post office with 40 people being potentially exposed. Forty blood samples were acquired to mimic samples that would have been obtained from the potentially exposed individuals.

Blood Collection, Exposure and Transportation

All donors were volunteers who willingly responded to an advertising call for participation in a research proposal approved by the Health Canada Research Ethics Board. In total, 40 blood samples were collected with informed consent by venipuncture into 4 mL lithium-heparnized Vacutainer tubes (Becton Dickenson, Oakville, ON, Canada) from six healthy volunteers (3 male, 3 female, ages 30-50), with no recent history of ionizing radiation exposure.

Irradiation was carried out on whole blood ex vivo in the Vacutainer collection tubes at room temperature using a ^{137}Cs Gammacell40 (Atomic Energy of Canada Ltd., Ottawa, ON, Canada) at a dose rate of 0.83 Gy min^{-1} calibrated by Fricke dosimetry by MDS Nordion (Ottawa, ON, Canada). The doses ranged between 0.0 and 4.0 Gy. To allow laboratory inter-comparisons, each laboratory received a set of 10 matched, irradiated samples that were coded by a third party to ensure the four laboratories could not identify the dose administered to the samples.

The samples were shipped to the participating laboratories in other cities by overnight courier express and were received the following day. One laboratory in close proximity to the coordinating laboratory sent a staff member to pick up their samples. During shipment, the blood tubes were surrounded by room temperature gel packs to ensure the blood was maintained at ambient temperature. An instruction form was sent with each shipment listing the samples enclosed and the contact information for reporting of dose estimates, as would occur in an actual emergency situation.

Sample Set-Up and Cell Culture

The samples were cultured by each laboratory using their own protocols, following recommended methods provided by IAEA (IAEA 2001) and ISO (International Organization for Standardization (ISO) 2008). Reagents suppliers varied between laboratories therefore sources are

not listed. Briefly, whole blood was diluted 1:9 with RPMI 1640 medium supplemented with 15-20% foetal bovine serum (FBS), 2 mM L-glutamine, 15-20 μM bromodeoxyuridine (BrdU) (except one lab who used 2.0 μg mL^{-1} cytochalasin-B addition to inhibit cytokinesis), 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and stimulated to divide with 1-2% phytohaemagglutinin (PHA). Cultures were incubated in a humidified atmosphere at 37ºC with 5% CO_2, for 48 h. Colcemid (10 μg mL^{-1}) was added to the cultures for the final 4 h to block mitosis, thus enriching the population of T-cells in metaphase. At 48 h after culture initiation, cells were treated with 75 mM KCl for 12-15 min and washed with Carnoy's Fixative (3 methanol: 1 glacial acetic acid) two to three times. Slides were made by dropping the resulting cell suspensions onto cold, wet slides or room temperature dry slides using a Hanabi-HS (Transition Technologies Inc, Japan) or by hand-dropping/steam-drying (depending on environmental conditions) to obtain optimal metaphase spreads for analysis. In those laboratories performing Fluorescence Plus Giemsa (FPG) staining, slides were stained with 20 μg mL^{-1} Hoechst 33258 (Bisbenzimide H 33258) for approximately 2 min, glass coverslips were then applied and the slides were exposed to UV light (365 nm) for 8 min. After rinsing several times with water, slides were stained with 10% Giemsa Gurr solution in buffer mixture at pH 6.8 for at least 10 min and then rinsed again with water. The slides were allowed to dry and were then mounted and sealed under glass coverslips with Permount.

Slide Scoring

All available trained scorers at each laboratory scored slides from each of the 10 samples for full triage DCA analysis and two laboratories (Lab A and B) also tested a new method called DCA QuickScan, devised by the Biodosimetry Laboratory at Atomic Energy of Canada Ltd (Chalk River, ON). For full triage DCA analysis, each scorer analyzed 50 metaphase spreads or 30 dicentrics,

ensuring that each metaphase spread had 46 centromeres (Lloyd et al. 2000). Depending upon the calibration curve of each laboratory, dicentrics or dicentrics plus centric rings were enumerated. The basis for the DCA QuickScan approach was that individual centromeres were not counted, but the metaphase spreads were rapidly examined for obvious damage. Metaphase spreads made with an environmental controlled slide making chamber, such as a Hanabi, was important for this method to produce complete, well spread metaphases that are consistent across each slide. After a quick examination (~10 seconds), if the metaphase spread appeared to be complete with no damage, then it was scored as normal and the scorer moved on to the next metaphase spread. If damage was observed (i.e. fragments, visible rings and/or dicentrics), the scorer carefully enumerated the damage, but the total number of chromosomes was not recorded. It was pre-determined that each dicentric had to be accompanied by an acentric fragment to reduce the chance of mistaking chromosomes with overlapping chromatids with a true dicentric. Using the DCA QuickScan approach, 50 metaphase spreads were examined unless five dicentrics were seen in less than 20 metaphase spreads.

Estimation of Dose

Dose response curves were generated using a weighted Poisson regression, $Y = c + \alpha D + \beta D^2$, where Y = the number of dicentrics per number of metaphase spreads scored, c = background value of dicentrics (and rings), D = radiation dose in Gy; and α and β are dose and dose-squared coefficients used to estimate the rate of dicentrics in metaphase spreads generated following IAEA guidelines (IAEA 2001). Maximum likelihood estimation was used to estimate the parameters of the fitted curves. Each of the four laboratories processed their own blood samples and reported their estimated doses based on using each laboratory's own calibration curve.

Chromosome Aberration Analysis Software (CABAS, Version 2.0), developed at the Swietokrzyska Academy, Kielce, Poland (Deperas et al. 2007) was used to fit the linear-quadratic dose-response relationships by the maximum likelihood method and to estimate the dose to the exercise samples.

RESULTS

The estimated doses by full triage DCA analysis by all scorers are depicted in Fig. 1. In this exercise, a total of 15 individuals participated from the four core laboratories of the CEN and the data represent a total of 150 biological dose estimates. For full triage DCA analysis, 83 % of the dose estimates were within ±0.5 Gy of the actual dose. Fig. 2 depicts the results from the same exercise, but with dose estimates derived with the DCA after only the first 10 cells were scored for each sample. In this case, only 61% of the dose estimates were within ± 0.5 Gy of the actual dose. Estimation of the dose was also made with the DCA after scoring the first 20 cells, where 74% of the dose estimates were within ± 0.5 Gy (Fig.3). Fig. 4a depicts the dose estimates as a function of the number of cells scored, while Fig. 4b depicts the dose estimates as a function of the number of dicentrics scored. This data was based on one representative scorer of a sample that received 2.4 Gy demonstrating how the confidence intervals on the dose estimates decrease with increasing numbers of cells and dicentrics scored. It can be observed that, in general, the dose estimate does not change significantly after ten cells or five dicentrics are scored.

Nine of the fifteen scorers from 2 laboratories also tested a new DCA scoring approach, termed DCA QuickScan. The results from this scoring approach are depicted in Fig. 5. Using this method, 80% of the dose estimates were within ± 0.5 Gy of the actual dose. When compared to the accuracy of the full triage-DCA with the same nine scorers, there was no appreciable difference in the

accuracy of the two methods. A summary of the results from the various DCA scoring scenarios is depicted in Table 1. For full triage DCA analysis, 17% of doses were not within ± 0.5 Gy (4% under-estimates, 13% over-estimates). Using the DCA QuickScan scoring approach, only 1% of the dose estimates were under-estimates.

The time required for analysis by each method was recorded by one laboratory and is depicted in Table 1. It was found that the average time required to score all 10 samples by one scorer was reduced from an average of 1265 min using full triage DCA to an average of 200 min using DCA QuickScan, with no appreciable reduction in accuracy.

DISCUSSION

In the current study, the capacity, efficiency and accuracy of the CEN were evaluated using several modified-DCA techniques. Ten coded, irradiated samples were sent to each of the four Canadian reference laboratories for biological dosimetry analysis. Using the DCA, cells were initially analysed for either 50 cells or 30 dicentrics (and rings) according to standard triage biological dosimetry recommendations (Lloyd et al. 2000), and dose estimates were calculated using DCA calibration curves at each laboratory. The full triage DCA approach was accurate (within ± 0.5 Gy of the actual dose) for 83% of dose estimates, but the time required to score using this approach was the longest.

As a strategy to decrease the scoring time required and increase the rate of sample turnover, dose estimates were derived using the DCA after only 10 and 20 cells were analyzed. Decreasing the number of cells scored reduced the scoring time required, but also reduced the accuracy. Scoring 20 cells by DCA reduced the accuracy to 73%, while scoring only 10 samples by DCA resulted in only

61% of dose estimates within ± 0.5 Gy of the actual dose applied.

As an alternative strategy for decreasing scoring time, the DCA QuickScan scoring approach was evaluated in two of the core laboratories. Nine of the 15 scorers in the CEN scored with the DCA QuickScan approach, resulting in 80% of doses estimates falling within ± 0.5 Gy of the actual dose. Of the estimates outside the ± 0.5 Gy range, only 1% were under-estimates. This scoring approach allowed the time of the scoring to be greatly reduced without compromising the accuracy of the dose estimations.

The DCA QuickScan approach is envisioned as a rapid screening approach, whereby initial quick dose estimates are made such that samples that were exposed to clinically significant doses can be prioritized for standard DCA scoring. Similarly, those samples exposed to lower doses (less than 1.5 Gy), can be identified with sufficient accuracy and deferred from full DCA analysis, thereby allowing scorers to focus on high-priority cases. Once the immediate emergency has passed or the more significant doses have been completed, these samples could be analysed by full DCA.

CONCLUSION

Through this exercise, Canada has demonstrated that it has a large capacity for triage-quality biodosimetry within the four core laboratories of the network. Although full triage DCA scoring was most accurate, it is recommended that for emergency situations samples should be pre-screened using the DCA QuickScan approach. This would quickly prioritize samples for full DCA analysis, thereby allowing biodosimetrists to focus their efforts on providing high accuracy dose estimates to those individuals that received clinically-significant radiological doses. Using this approach, it is feasible to produce initial dose estimates for 150 individuals within a few hours of the samples being processed. The next study will aim to further evaluate DCA QuickScan by generating full dose response curves

from which the accuracy and the sensitivity will be determined. From these data the minimum number of metaphase spreads required to confidently identify samples which received less than 1.5 Gy can be established.

Overall, this exercise demonstrated an increased capacity for performing the DCA for biological dosimetry, not only through an increasing number of qualified scorers but also through new scoring strategies. It also demonstrated the operability of the network and its ability to provide timely dose estimates for a large number of exposed individuals.

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FIGURES

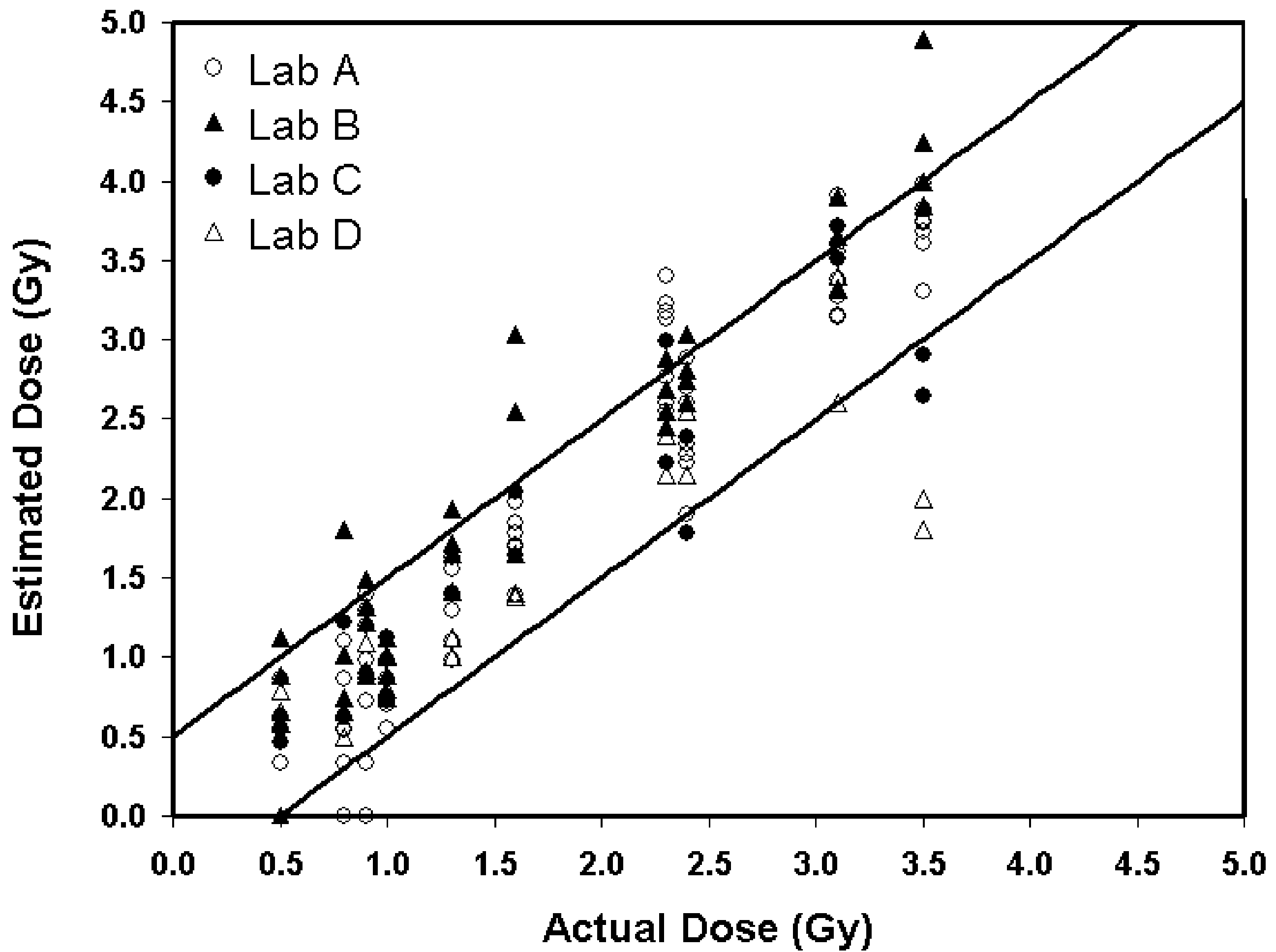


Fig. 1. Estimated doses by full triage DCA analysis scored by all scorers in each laboratory. Each data point represents the dose estimate from one scorer, with scorers from the same laboratory shown using the same symbol. The solid lines represent ± 0.5 Gy intervals.

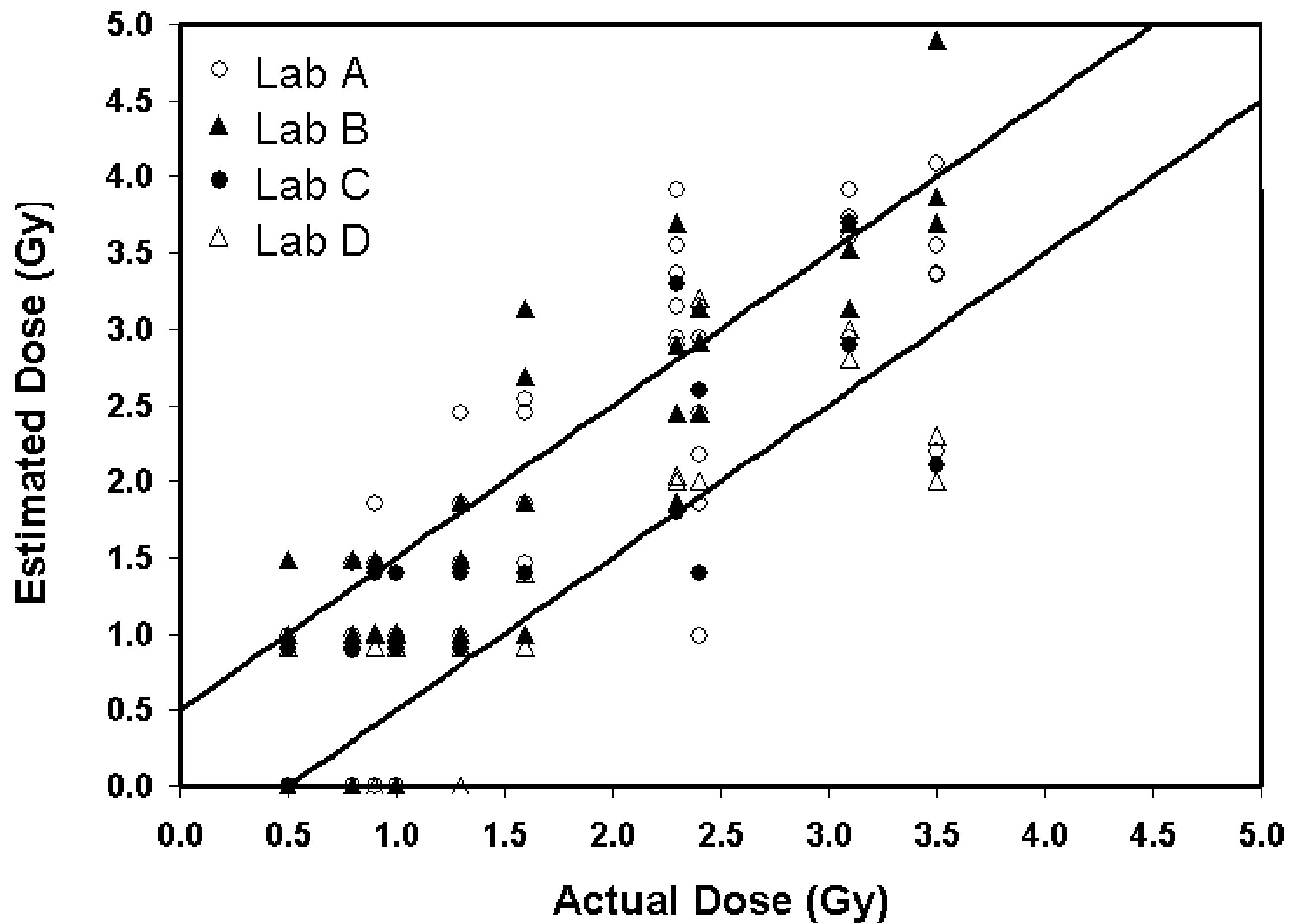


Fig. 2. Estimated doses by triage DCA analysis after enumerating 10 metaphase spreads. Each data point represents the dose estimate from one scorer, with scorers from the same laboratory shown using the same symbol. The solid lines represent ± 0.5 Gy intervals.

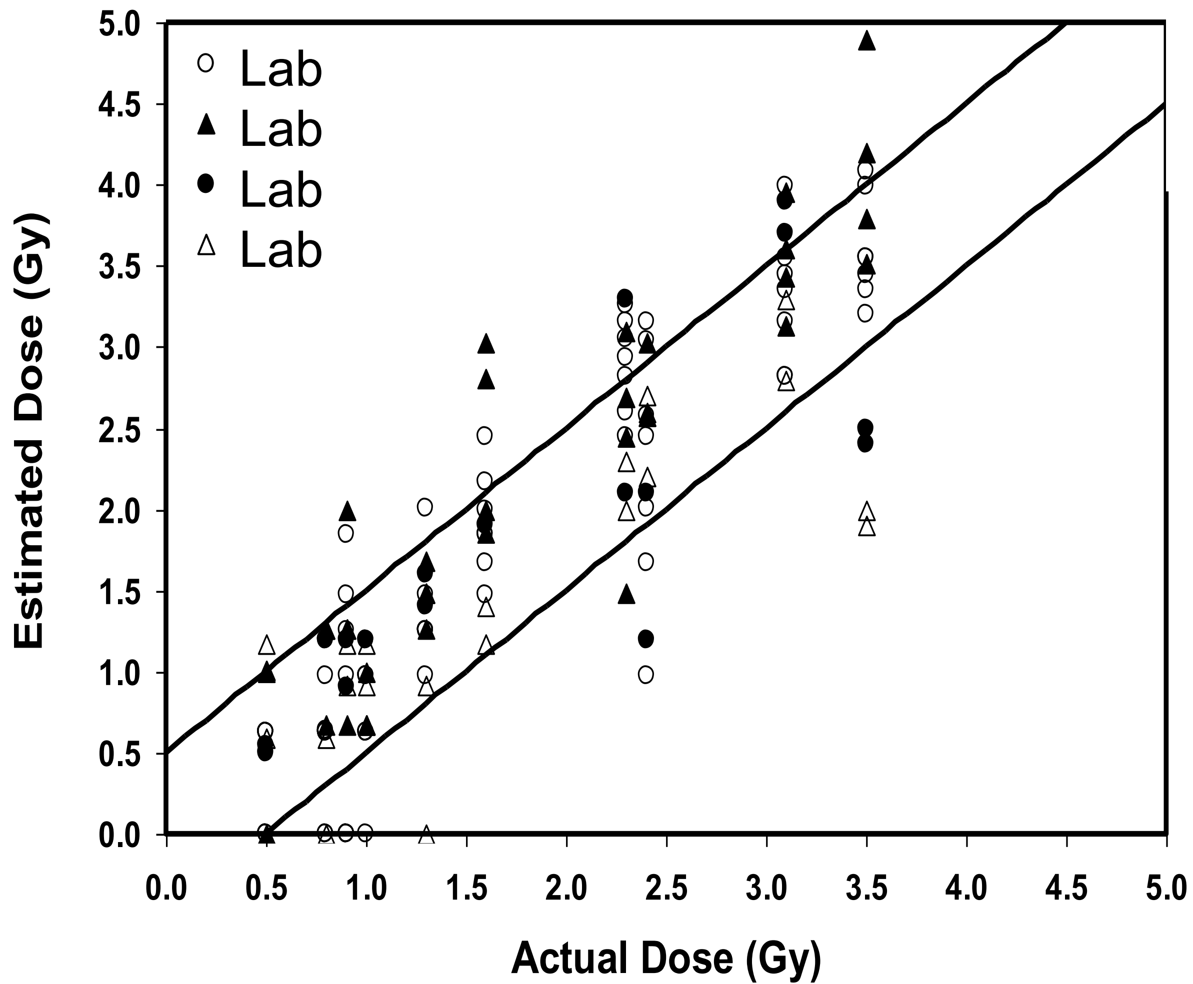
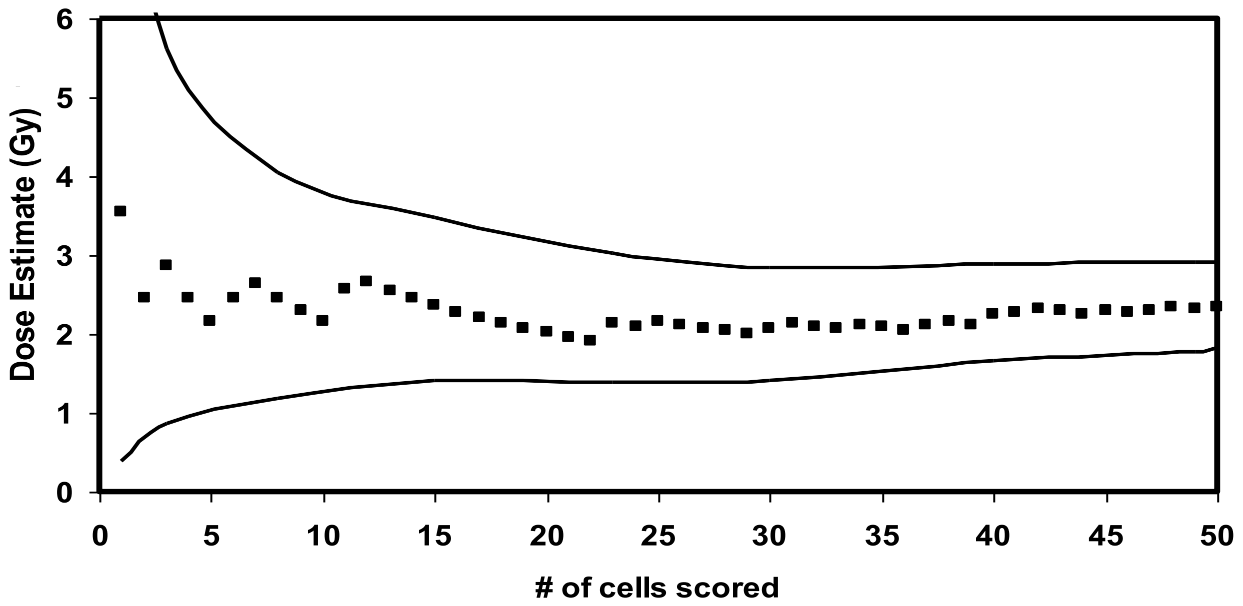


Fig. 3. Estimated doses by triage DCA analysis after enumerating 20 metaphase spreads. Each data point represents the dose estimate from one scorer, with scorers from the same laboratory shown using the same symbol. The solid lines represent ± 0.5 Gy intervals.



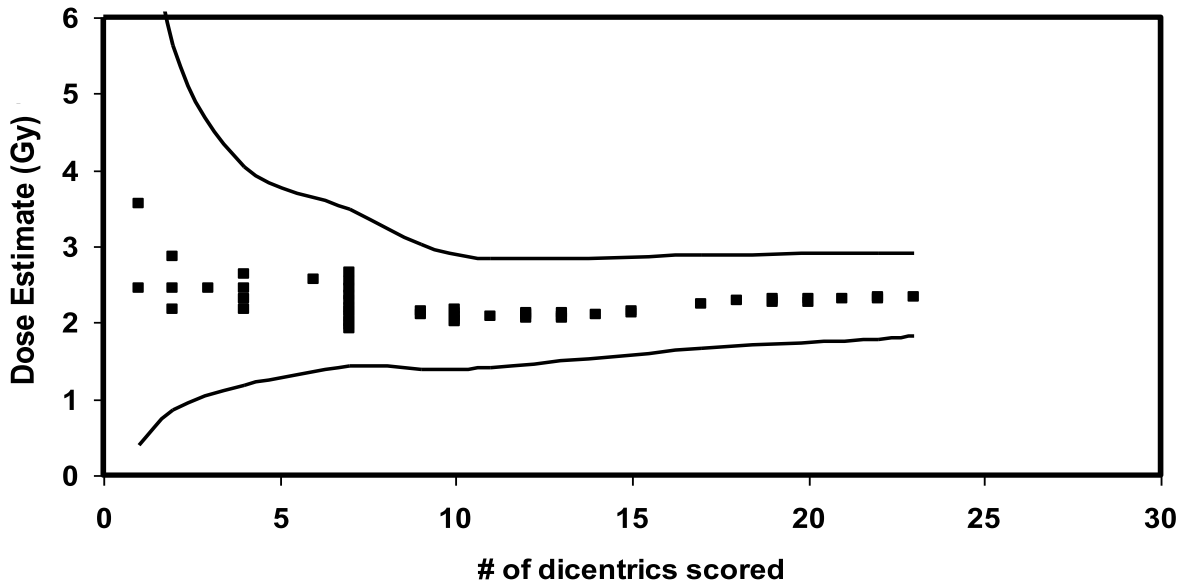


Fig. 4 a/b. Dose estimates by triage DCA as a function of the number of cells scored and as a function of the number of dicentrics scored. The symbols represent the dose estimates and the solid lines represent the 95% confidence intervals on the dose estimates. The data are based on one representative scorer of a sample that received 2.4 Gy.

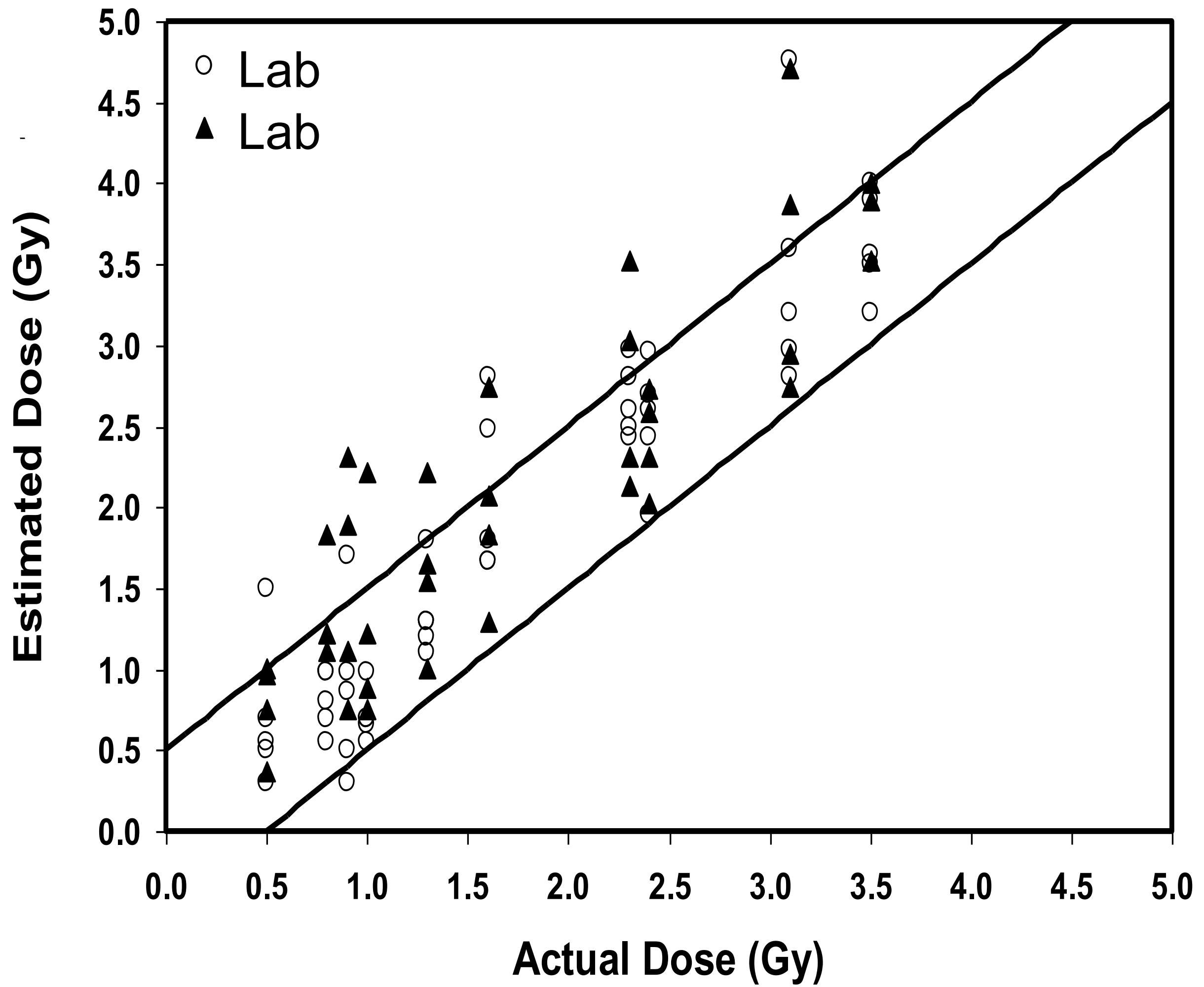


Fig. 5. Estimated doses by DCA QuickScan analysis. Each data point represents the dose estimate from one scorer, with scorers from the same laboratory shown using the same symbol. The solid lines represent ± 0.5 Gy intervals.

Table 1. Comparison of scoring methods

Method	% within 0.5 Gy	% over estimates	% underestimates	Ave. time to score 10 slides (min)
Full Triage	83%	13%	4%	1265
20 spreads	74%	14%	12%	500
10 spreads	61%	24%	15%	250
Quick Scan	81%	18%	1%	200